Effect of acute physical exercise on motor sequence memory

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ABSTRACT

Recent studies suggest that acute physical exercise improves memory functions by increasing neural plasticity in the hippocampus. In animals, a single session of physical exercise has been shown to boost AEA (anandamide), an endocannabinoid known to promote hippocampal plasticity, which may in turn benefit hippocampal-dependent learning. Hippocampal neuronal networks do not only encode episodic memory representations, but also contribute to the sequential organization of memory elements, including motor sequences (Schendan *et al.*, 2003; Eichenbaum, 2017). While previous work established that acute physical exercise has positive effects on declarative memory, whether it also influences memory for motor sequences remains unresolved.

Here we studied the impact of moderate and high intensity acute physical exercise on motor sequence learning, and its underlying neurophysiological mechanisms in humans. To this end, we acquired behavioral, fMRI and AEA level data in fifteen healthy participants across three visits while they performed a serial reaction time task (SRTT) before and after a period of exercise (moderate or high intensity) or rest.

We report that physical exercise increased AEA levels, and activity in the right hippocampus and caudate nucleus. Activity in both areas was directly linked to SRTT performance, which itself correlated with circulating AEA levels. These findings support that acute physical exercise favors hippocampal plasticity and potentially its broader role in memory function, including the consolidation of motor sequences.

INTRODUCTION

From typing on our smartphones to tying our shoes, motor skills are a crucial part of our everyday activities. It is affected in many neurological disorders such as ataxia (a deficit in motor coordination) or Parkinson's disease. Motor skill learning requires both explicit and implicit mechanisms, involving both hippocampal and striatal activity (Albouy *et al.*, 2008; Marinelli *et al.*, 2017). Studies in patients have shown that hippocampal damage impairs both explicit and implicit memory (Addante, 2015). Conversely, regular physical exercise has been shown to promote synaptic plasticity in the hippocampus (Uysal *et al.*, 2005; Pereira *et al.*, 2007; Wu *et al.*, 2007; Erickson *et al.*, 2011), while also enhancing learning and memory in general (Roig *et al.*, 2013). Regarding motor skill learning specifically, lower fitness levels have been linked to worse performance on motor sequence learning task (Pontifex *et al.*, 2014).

Much like regular exercise, single bouts of physical exercise may also benefit diverse cognitive functions such as executive functions (Yanagisawa *et al.*, 2010) but also hippocampal plasticity and function, including declarative memory in humans (van Dongen *et al.*, 2016; Marin Bosch *et al.*, 2017). Acute physical exercise increases the levels of endocannabinoids in the brain, which can be measured peripherally (Sparling *et al.*, 2003; Dietrich & McDaniel, 2004). Specifically, higher levels of the endocannabinoid anandamide (AEA) have been positively linked to an increase in plasticity and in cannabinoid receptor 1 (CB1) activity in the hippocampus (Hill *et al.*, 2010; Tantimonaco *et al.*, 2014) through LTP (Carlson *et al.*, 2002). The hippocampus is not the only brain region modulated by CB1 activity. Recent studies have shown that CB1 activity protects striatal neurons in culture against excitotoxicity (Blazquez *et al.*, 2015) and that it is reduced in striatal neurons in a mouse-model of ataxia (Rodriguez-Cueto *et al.*, 2017). Consistent with enhanced striatal function, one report indicated that acute physical exercise also improved procedural memory in a visuomotor accuracy tracking task in a dose-response manner, with higher intensity being significantly more beneficial than lower intensity

(Thomas *et al.*, 2016). Finally, a recent study reported that overnight consolidation of motor sequence learning was impaired in hippocampal amnesiacs compared to matched controls, despite similar initial learning (Schapiro *et al.*, 2019), hence further stressing that the hippocampus critically contributes to the consolidation of not only declarative memory but also motor sequences. Based on these observations from different research fields, we hypothesized that one bout of exercise should promote hippocampal (and striatal) activity and functions, plausibly via increased circulating levels of AEA, and thus enhance the consolidation of memory for motor sequences. We also predicted that higher (compared to moderate) exercise intensity would yield stronger effects.

To test these hypotheses, we combined behavioral, imaging, and blood samples measurements in an experimental protocol including two conditions of exercise with different intensities (one below and one above the ventilatory threshold) and one additional no-exercise or rest condition (Fig. 1A). We tested 15 right-handed, healthy and fit males (23.2 ± 4.21 years old) in a randomized within-subjects design involving three visits (one condition per visit). To assess motor sequence learning, we adapted a finger tapping task or serial reaction time task (SRTT) previously used by Ros et al. (2014), in which participants were trained at executing a sequence of keypresses with the four fingers of their non-dominant left hand as indicated by visual cues on a screen (Fig. 1C). Participants were not instructed or told explicitly the fact that they produced a fixed sequence of 12 button presses (a different sequence was trained on each visit), repeated 10 times in each training block. Thus, at each visit, participants came in the morning and performed the first session (Session 1) of the motor sequence task in the MRI. Session 1 was composed of four blocks (three sequence blocks and one random block, in position 2, Fig. 1B). Then, participants rested for 30 minutes, or cycled at moderate intensity for 30 minutes, or cycled at high intensity for 15 minutes. Blood samples were taken right before and right after resting or cycling (see Fig. 1A for full protocol). Forty-five minutes after the

second blood sample, i.e. once heart rate and breathing came back to baseline, participants entered the MRI again to perform the second session (Session 2) of the SRTT in the MRI (see Methods section for full description). The effects of exercising were thus assessed by comparing, for each participant, the performance improvement from Session 1 to Session 2 from each exercise condition.

RESULTS

Behavioral

Participants were instructed to be as quick and as correct as possible on each trial of the SRTT, but there was no time limit. To account for speed-accuracy trade-off, we defined performance as the percentage of correct trials per block divided by the average reaction time on that block. Exercise-related SRTT improvement or consolidation was computed as the difference in performance from Session 1 to Session 2. On one hand, using separate one-sample t-tests against zero, we found that participants' performance improved only for the sequence blocks, but not for the random blocks (rest sequence: $T(14)=2.40$, $p=0.031$, rest random: $T(14)=-0.95$, $p=0.36$, moderate sequence $T(14)=3.74$, $p=0.002$, moderate random $T(14)=0.39$, $p=0.70$, high sequence $T(14)=3.76$, $p=0.002$, high random $T(14)=1.72$, $p=0.11$). On the other hand, these data were analyzed using repeated-measures ANOVA with Exercising Condition (rest, moderate and high intensity exercise) and Block type (sequence, random) as within-subjects factors. We report an effect of Exercising Condition ($F(2,28)=3.36$ $p=0.049$) and an effect of Block type ($F(2,28)=16.28$ $p=0.001$), with no interaction between these effects ($F(2,28)=0.43$, $p=0.655$; Fig. 2). Post-hoc analyses on the effect of Exercising Condition showed a significant difference between rest and high ($p=0.049$) and a tendency towards significant difference between rest and moderate ($p=0.069$), while moderate and high were not significantly different ($p=0.558$). Due to the combination of results we performed exploratory post-hoc

analysis on the non-significant interaction between the effect of Exercising Condition and the effect of Block Type. This analysis revealed no difference between random and sequence blocks in the Rest condition (p=0.137), while the difference between random and sequence blocks was significant for both moderate (p=0.009) and high (p=0.007) intensity Exercising conditions.

Functional MRI

After standard preprocessing and corrections for breathing and heart rate, the fMRI data from each participant acquired after each exercising condition were analyzed using an event-related general linear model (see Methods section for detailed description). Event-onsets for the sequence and random blocks were defined as the moments when one of the dots on the screen turned into a star. The inverse reaction time (-RT) of each trial was added as parametric modulator of the main event regressors. A flexible factorial model was then run with Subject and Block type (sequence and random) using the contrast images to the main effects of the sequence and random blocks from each participant. To identify common motor activations between the sequence and random blocks we conducted a conjunction analysis across all subjects. This analysis yielded activations in the right precentral gyrus (contralateral to hand used in the task) and left cerebellum, which is consistent with previous literature for this type of task (Hardwick *et al.*, 2013) (Supplementary Table 1). We then tested for global differences in activity elicited after exercise (moderate and high) versus rest for the sequence blocks and found increased bilateral activation of the precuneus (peaks at: [-16 -68 30] and [20 -60 22]). To directly address one of the main hypotheses that better performance after exercise would rely on hippocampal and striatal circuitry, we looked at the effect of the parametric modulator (-RT) to reveal any regions whose activation increases selectively for faster correct trials, and more so after exercise (moderate and high) than after rest. We found such an effect in the right hippocampus (peak at [30 -6 -24], Fig. 3A) and in the right caudate (peak at [20 24 2], Fig. 3B).

Activations in both regions survived small volume correction (cluster level: $p_{right\ hippocampus}$=0.002, $p_{right\ caudate}$=0.020; peak level: $p_{right\ hippocampus}$=0.003, $p_{right\ caudate}$=0.038) using predefined regions of interest (see Methods). More importantly, these activations are nowhere to be found when looking at this same contrast but only on the random blocks. See supplementary Table 1 for all activations.

Anandamide

Blood samples were taken before (baseline) and after each exercising condition. Analyses on the difference between the second and the first baseline sample revealed a significant effect of Exercising Condition on AEA levels ($F(2, 28)=41.991$, $p<0.001$), with increased AEA after moderate and high intensity exercise compared to after rest ($p_{mod-rest}<0.001$; $p_{high-rest}<0.001$) and no difference between both exercising conditions ($p_{high-mod}=0.080$; Fig. 4A).

Additionally, we found a positive correlation between AEA increase and performance for both the moderate ($R=2.397$ $p=0.032$, Fig. 4B) and for the high ($R=2.606$ $p=0.022$, Fig. 4C) intensity exercising conditions. Please note that this was not the case for random blocks, suggesting that increase in AEA specifically enhanced performance for the sequence blocks. Finally, as exploratory analyses, we tested whether changes in AEA levels correlated with hippocampal or caudal activations, but did not observe any such correlation (all $p> 0.05$).

DISCUSSION

This study shows that acute physical exercise at both moderate and high intensities increases AEA levels and hippocampal and striatal recruitment during SRTT, with benefits for motor learning performance.

At the behavioral level, participants successfully learned the sequence, as shown by their better performance for sequence compared to random blocks. We also observe a significant

overall effect of acute physical, which shows a clear benefit from engaging in moderate or even high intensity exercise rather than resting. This benefit comes from both the sequence and random blocks, suggesting the impact on discriminating visuo-motor processes overall. However, the performance improvement on the random blocks are not significantly different from zero in any of the Exercising Conditions, meaning that participants did not improve significantly after any of the rest or exercising sessions. This alludes to the specific impact on memory processes and not only general visuo-motor ones. Due to the duality of the processes we have seen to be involved and impacted, we conducted the exploratory post-hoc analysis on the interaction between the effect of Exercising Condition and the effect of Block Type. With this analysis we sought to disentangle the effect on memory processes from that on visuo-motor processes in each Exercising Condition. This analysis showed significant performance improvement for the sequence blocks only after acute exercise (moderate and high) but not after the rest condition, thus supporting that acute physical exercise may promote the consolidation of motor sequence memory.

Results from the existing literature show some inconsistencies, with acute exercise improving motor skill learning in a within-subjects study (Mang *et al.*, 2016), and in a dose-dependent manner in a between-subjects study (Thomas *et al.*, 2016), but did not have an effect in a different between-subjects study (Singh *et al.*, 2016). Please note that between-subjects designs may not have provided sufficient sensitivity for this type of manipulation. In our within-subjects study, we tested two very carefully calibrated exercise intensities, either below or above the ventilatory threshold. While high intensity exercise yielded numerically better performance, we did not report a statistically significant difference between moderate and high intensity exercise, but an overall exercising effect. Most studies showing an effect of acute physical exercise on motor skill learning only compare one intensity (which is usually high) to rest (refs). The previously mentioned study by Thomas et al. (2016) is one of the few comparing

more than one exercise intensity to rest. However the comparison is made between exercise at 45% and 90% of the maximum power output (a measure comparable to HRmax), which is arguably comparing low intensity (for reference our Rest condition already corresponds to 30-35% of HRmax) to very high intensity. We agree with their statement that exercise intensity plays a crucial role; we however speculate it may more in a step-wise manner, rather than a progressive dose-dependent increase.

At the brain level, we found increased bilateral precuneus activity for both the moderate and high intensity exercising conditions as compared to the rest condition, consistent with the role of the precuneus in motor tasks requiring coordination and/or imagery (Hanakawa *et al.*, 2003; Wenderoth *et al.*, 2005). Next, we report that activity in the right hippocampus and caudate nucleus covaried with behavioral performance on the SRTT (faster correct trials) after both moderate and high intensity exercise (compared to rest), thus directly confirming our main initial hypothesis that hippocampal and striatal circuits contribute to exercise-related performance gains. No such relationship was found for the random sequences, further suggesting that these regions contributed to motor sequence learning, beyond simple visuo-motor mapping,

These results are consistent with motor sequence learning incorporating both implicit and explicit learning components, implicating striatal and hippocampal regions (Schendan *et al.*, 2003; Poldrack *et al.*, 2005). Moreover, a positive relationship between activity in the striatum and hippocampus and motor skill learning was also observed in Parkinson's patients and healthy controls after a 3-month long exercising protocol (Duchesne *et al.*, 2016). While the results of our study suggest that one single session of exercise may impact the same brain regions as long-term regular exercise, the exact mechanisms underlying the observed brain-behavior relationships are likely to differ (Lohse *et al.*, 2014). Specifically, in our case, activity in both regions appeared to contribute to response times for accurate motor output, while in Duchesne et al. (2016) this effect was only seen in Parkinson's patients. The lack of change in

healthy controls after training was explained by a ceiling effect, which did not leave much room for improvement.

Here we additionally tested whether changes in SRTT performance may relate to exercise-related changes in endocannabinoids' levels (i.e. AEA). First, we replicated a significant AEA increase in response to physical exercise (Sparling *et al.*, 2003; Dietrich & McDaniel, 2004) (while levels slightly dropped after rest, as expected from metabolic and circadian fluctuations (Valenti *et al.*, 2004; Murillo-Rodriguez *et al.*, 2006)). Second, our results revealed a positive correlation between AEA levels and SRTT performance for both the moderate and for the high intensity exercising conditions, suggesting that sequence learning may have benefited from exercise also via AEA increase. Please note that while both AEA increase and higher hippocampal/striatal activity were linked to increased SRTT performance (for the sequence Block in the moderate and high Exercising Conditions), this activity did not correlate with AEA levels (all $p>0.05$). Because of such joined relationship with performance and because the existing animal literature strongly supports that AEA promotes plasticity in both the hippocampus and the striatum (Carlson *et al.*, 2002; Thompson & Perkel, 2011; Cassilhas *et al.*, 2016), we cannot rule out that increased AEA affected hippocampal and/or striatal function in our experiment, despite the absence of a simple linear relationship between both measurements. Moreover, AEA is the strongest endogenous agonist of the CB1 system (Scherma *et al.*, 2019), which itself has known neuroprotective effects (Carvalho *et al.*, 2017). Altogether, the data from the present study support that the preventive role of physical exercise against cognitive decline. Pharmacotherapies targeting the AEA or CB1 system may also benefit people unable to perform physical activity on their own due to disease, disability or old age.

To conclude, here we report a robust and positive impact of one bout of physical exercise on motor sequence learning. Specifically, we demonstrate that exercise enhanced hippocampal

and striatal function and increased endogenous endocannabinoids levels, and that both contributed to better behavioral performance.

METHODS

Participants

Twenty-one healthy young males between the ages of 18 and 35 participated in this study, for which they were financially compensated. This study was approved by the Ethics Committee of the Geneva University Hospitals. All participants included in this study were right-handed, non-smokers, without psychiatric or neurological history, and had a normal or corrected-to-normal vision. None of the participants had any musical training, which may imply the learning of finger motor sequences, potentially similar to the SRTT. Participants scored in normal ranges on self-assessment questionnaires for depression (BDI (Steer *et al.*, 1997)), anxiety (STAI (Spielberger CD, 1983)), and circadian typology (PSQI (Horne JA, 1976)). They also reported exercising on a regular basis (at least twice a week) and participants' VO2max were all within the 40-65 ml/kg/min range. This yielded a homogenous sample of regularly exercising young men. One participant had to be excluded for non-compliance and five were excluded due to technical difficulties.

Concerning the sample size, please note that although 15 participants may appear at the lower limits, here we used a carefully controlled cross-over within-subjects design, which is by essence less affected by inter-individual variability than between-subjects studies. Between-subjects studies typically require 4 to 8 times more participants than within-subjects designs (Bellemare *et al.*, 2014), and even with half the number of participants within-subjects studies are much more powerful than their between-subjects counterpart (Thompson & Campbell, 2004). Based on the effect sizes from a previous behavioral study using the same SRTT as administered here, we determined that the minimum sample size of a within-subjects design for

a targeted power of over 0.95, was 6 (Ros *et al.*, 2014). Concerning the fMRI analyses, based on previous data from previous studies (Schendan *et al.*, 2003; Poldrack *et al.*, 2005) we established that the minimum sample size for activation in the hippocampus and/or striatum was 7.

Experimental procedure

Participants came to the lab for a total of four visits; one introductory (visit 0) and 3 experimental (visits 1, 2 and 3).

Visit 0

Participants performed a VO2max procedure (detailed below), as well as a demo session of the serial reaction time task (SRTT). Only those participants with a VO2max between 40 and 65 ml/kg/min were called back and invited to complete the rest of the study. These VO2max limits were set specifically so that only physically trained participants but not elite athletes were included.

Visits 1, 2 and 3

These visits were spaced between one and two weeks, and since this was a within-subjects protocol, exercising conditions and task versions (see below) were fully counterbalanced.

Participants were instructed to keep a regular exercising schedule during the week before each visit, and their exercising schedule was documented using a fitness tracker (Fitbit Charge HR, Fitbit, San Francisco, USA). They were also instructed to avoid intense physical activity during the 48 hours preceding each experimental visit.

For each visit the same schedule was followed, starting at 8:00 AM and ending at 12:00 PM (Figure 1A). Participants first had a standardized breakfast in the lab including black coffee or

tea, orange or apple juice, bread and jam. They were allowed to have as much of these items as they wanted as soon as they would have the same amount on all visits. This breakfast minimized fat intake, because this has the potential to affect circulating levels of the endocannabinoid anandamide (AEA). One hour and a half later, participants were placed in the MRI scanner and performed the first session of the SRTT, after which a medical doctor took the first blood sample.

Participants were then asked to rest or exercise while wearing a Polar RS800CX N (Polar RS 800 CX, Polar, Finland) to measure heart rate. In the case of exercise, participants pedaled on a cycle ergometer (Ergoline GmbH, Bitz, Germany), maintaining a pedaling frequency between 60 and 80 cycles per minute. The exercise intensities were determined by adapting the load on the ergometer so as to match 60% (moderate intensity) or 75% (high intensity) of the participant's maximum heart rate. For the 'rest' condition, participants sat on a chair and were allowed to look through selected magazines. Shortly after the end of the exercising or rest period, a second blood sample was taken. To allow for breathing and heart rates to go back to baseline, participants waited for 45 minutes before they were placed back in the MRI for the second session of the SRTT.

Serial Reaction Time Task (SRTT)

We used the SRTT task described in Ros et al. (2014). Within each visit, the SRTT was divided into two sessions composed by 4 blocks each (Fig. 1B). Participants were shown a black screen with four white horizontally-organized dots corresponding to their index, middle, ring and little fingers of their non-dominant hand (see Fig. 1C). Each finger was placed over a button of a 4-button MRI-compatible box (HH-1x4-CR, Current Designs Inc.). When one of the dots turned into a star, the participants had to press the corresponding button as fast as they could. As soon as they pressed a button, another dot would turn into a star and they had to then press the button associated to the new star.

For sequence blocks, unbeknownst to them, the button presses formed a sequence of 12 presses, or trials, repeated 10 times in a block. Random blocks were designed to match sequence blocks in the number of digit presses and digit span between trials, but there was no underlying repeated sequence. One block was formed of 120 trials, and each session comprised 4 blocks. For each session, the second block was the random block. Participants did not spontaneously report to have noticed any repetition during the sequence blocks. For Visits 1, 2 and 3, there was one session before and one after the exercising condition (Fig. 1A). For each exercising condition, one distinct 12-item sequence (or version of the task) was trained, and we did not observe any significant effect of task version on SRTT performance ($F(2,28)=0.690$ $p=0.510$).

During Visit 0 participants did a small introductory session of the task (demo version), where all trials were random and feedback was given after every trial (correct or not).

Behavioral analysis

The program Statistica (Version 12, www.statsoft.com, StatSoft, Inc. TULSA, OK, USA) was used for all behavioral analyses performed in this study. Performance was defined as accuracy (described as percentage of correct responses) divided by reaction time (mean reaction time per correct trial). We ran our analyses on the difference in performance from Session 1 to Session 2, that is, a t-test of each variable against zero and an ANOVA with Exercising Condition (rest, moderate intensity, high intensity) and Block type (random, sequence) as factors. All post-hoc analyses were Newman-Keuls tests and all correlations were Spearman Rank Order correlations.

VO2max measure

During Visit 0, all participants performed a maximal incremental test on an electrically braked cycle ergometer (Ergometrics er800S, Ergoline, Jaeger, Germany). Using a metabolic

unit (K4b^2, Cosmed, Italy), respiratory gas flow and ventilation were measured breath-to-breath. This metabolic unit was composed of a Zirconium Oxygen analyzer, an infrared CO2 meter and a turbine flowmeter. The gas analyzers and the turbine were calibrated as recommended by the manufacturer, the former with ambient air and with a mixture of known gases (O_2 16%, CO_2 5%, N_2 as balance), and the latter using a 3-L syringe. Heart rate was monitored on a beat-by-beat basis using cardiotachography (Polar RS 800 CX, Polar, Finland).

The following gas exchange variables were recorded on a breath-by-breath basis: V˙O2, V˙CO2, V˙E, and RER. They were then averaged over 10 –second sliding intervals for later analysis. The test started with participants pedaling at a power output of 50W for 4 minutes. Power was increased by 25 W every 2 minutes until achieving 80% of maximal HR predicted by age. From this point forward, power was increased by 25W every 1 minute until volitional exhaustion. Pedaling frequency had to be maintained in the range of 60 - 80 cycles per minute throughout the test.

VO2max was determined when the following criteria were fulfilled: RER > 1.1, plateau in VO2 (change of <100 mL·min^{-1} in the last three consecutive 20-s averages), and a HR less than 10 beats·min-1 away from the calculated maximal level predicted by age. The results from this test were used to select the appropriate power output for the experimental visits, based on the relationship between power output and VO2.

Functional MRI data acquisition and analysis

A 3 Tesla MRI scanner (SIEMENS Trio® System, Siemens, Erlangen, Germany) with a 32-channel head coil was used to acquire MRI data. We acquired T2*-weighted fMRI 2D images with a multiband echo-planar sequence, which acquires 3 slices at a time using axial slice orientation (66 slices; voxel size, 2 x 2 x 2 mm; repetition time (TR) = 1880 ms; echo time (TE) = 34 ms; flip angle (FA) = 60°). The last sequence of Visit 1 was a T1-weighted 3D sequence (192

contiguous sagittal slices; voxel size, 1.0 x 1.0 x 1.0 mm; TR = 1900 ms; TE = 2.27 ms; FA = 9°), which provided a whole-brain structural image.

We used SPM12 (Wellcome Department of Imaging Neuroscience, London, UK) for the analysis of the functional images. Preprocessing followed standard procedures: realignment, slice timing to correct for differences in slice acquisition time, normalization (to an MNI template), and smoothing (with an isotropic 8-mm FWHM Gaussian kernel). Corrections to regress out potential artifacts coming from heart rate and breathing were performed using Retroicor (Gary H. Glover, 2000) and RVHcorr (Birn *et al.*, 2006; Birn *et al.*, 2008).

We included the parametric modulator –RT which corresponds to the reaction time of each trial multiplied by -1, so that any increase in MRI signal would be linked to faster reaction times. We focused on this parametric modulator and used the contrast exercise > rest (i.e. moderate and high together minus 2*rest), including all sequence blocks in the second session of every visit, and found activations in the right hippocampus and right caudate. We defined an image of the right hippocampus using the AAL atlas in the WFU PickAtlas toolbox version 2.4 (Maldjian *et al.*, 2003). We used this image to perform small volume correction on the activations seen for the Exercise > Rest contrast. We followed the same correction procedure for the right caudate.

As quality checks, we computed the main effect during sequence blocks and the main effect during the random blocks separately. To test whether both block types elicited similar pattern of activity, we ran a flexible factorial design on the contrast images corresponding to these main effects, using subjects and block type (sequence vs random) as factors. We then performed a conjunction analysis on all our participants and obtained the common activations shown in Supplementary Table 1 including right motor cortex and left cerebellum.

Blood samples

Before and after each exercising condition, 2.5 mL of blood were collected into a BD Vacutainer K_2EDTA 5.4 mg tube. This tube was immediately centrifuged for 10 minutes at 8009g at 4°C, the supernatant (plasma) was taken in aliquots of 200 µL. All samples were then frozen and stored at -80°C until analysis.

The endocannabinoid AEA levels were determined from 100 µl of plasma by liquid-liquid extraction. This was followed by liquid chromatography (Ultimate 3000RS, Dionex, CA, USA) and mass spectrometry using a 5500 QTrap® triple quadrupole/linear ion trap (QqQLIT) mass spectrometer equipped with a TurboIon-SprayTM interface (AB Sciex, Concord, ON, Canada) as described previously (Thomas *et al.*, 2009; Quercioli *et al.*, 2013) .

REFERENCES

Addante, R.J. (2015) A critical role of the human hippocampus in an electrophysiological measure of implicit memory. *NeuroImage*, **109**, 515-528.

Albouy, G., Sterpenich, V., Balteau, E., Vandewalle, G., Desseilles, M., Dang-Vu, T., Darsaud, A., Ruby, P., Luppi, P.H., Degueldre, C., Peigneux, P., Luxen, A. & Maquet, P. (2008) Both the hippocampus and striatum are involved in consolidation of motor sequence memory. *Neuron*, **58**, 261-272.

Bellemare, C., Bissonnette, L. & Krrger, S. (2014) Statistical Power of within and Between-Subjects Designs in Economic Experiments. *SSRN Electronic Journal*.

Birn, R.M., Diamond, J.B., Smith, M.A. & Bandettini, P.A. (2006) Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *NeuroImage*, **31**, 1536-1548.

Birn, R.M., Smith, M.A., Jones, T.B. & Bandettini, P.A. (2008) The respiration response function: the temporal dynamics of fMRI signal fluctuations related to changes in respiration. *Neuroimage*, **40**, 644-654.

Blazquez, C., Chiarlone, A., Bellocchio, L., Resel, E., Pruunsild, P., Garcia-Rincon, D., Sendtner, M., Timmusk, T., Lutz, B., Galve-Roperh, I. & Guzman, M. (2015) The CB(1) cannabinoid receptor signals striatal neuroprotection via a PI3K/Akt/mTORC1/BDNF pathway. *Cell Death Differ*, **22**, 1618-1629.

Carlson, G., Wang, Y. & Alger, B.E. (2002) Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nat Neurosci*, **5**, 723-724.

Carvalho, T.G., Doria, J.G. & Ribeiro, F.M. (2017) mGluR5, CB1 and neuroprotection. *Oncotarget*, **8**, 3768-3769.

Cassilhas, R.C., Tufik, S. & de Mello, M.T. (2016) Physical exercise, neuroplasticity, spatial learning and memory. *Cell Mol Life Sci*, **73**, 975-983.

Dietrich, A. & McDaniel, W.F. (2004) Endocannabinoids and exercise. *British journal of sports medicine*, **38**, 536-541.

Duchesne, C., Gheysen, F., Bore, A., Albouy, G., Nadeau, A., Robillard, M.E., Bobeuf, F., Lafontaine, A.L., Lungu, O., Bherer, L. & Doyon, J. (2016) Influence of aerobic exercise training on the neural correlates of motor learning in Parkinson's disease individuals. *Neuroimage Clin*, **12**, 559-569.

Eichenbaum, H. (2017) Memory: Organization and Control. *Annu Rev Psychol*, **68**, 19-45.

Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E. & Kramer, A.F. (2011) Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 3017-3022.

Gary H. Glover, T.-Q.L., David Ress (2000) Image-based method por retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magnetic Resonance in Medicine*, **44**, 162-167.

Hanakawa, T., Immisch, I., Toma, K., Dimyan, M.A., Van Gelderen, P. & Hallett, M. (2003) Functional properties of brain areas associated with motor execution and imagery. *J Neurophysiol*, **89**, 989-1002.

Hardwick, R.M., Rottschy, C., Miall, R.C. & Eickhoff, S.B. (2013) A quantitative meta-analysis and review of motor learning in the human brain. *NeuroImage*, **67**, 283-297.

Hill, M.N., Titterness, A.K., Morrish, A.C., Carrier, E.J., Lee, T.T., Gil-Mohapel, J., Gorzalka, B.B., Hillard, C.J. & Christie, B.R. (2010) Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. *Hippocampus*, **20**, 513-523.

Horne JA, O.O. (1976) A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. International journal of chronobiology. *International journal of chronobiology* 97-110.

Lohse, K.R., Wadden, K., Boyd, L.A. & Hodges, N.J. (2014) Motor skill acquisition across short and long time scales: a meta-analysis of neuroimaging data. *Neuropsychologia*, **59**, 130-141.

Maldjian, J.A., Laurienti, P.J., Kraft, R.A. & Burdette, J.H. (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*, **19**, 1233-1239.

Mang, C.S., Snow, N.J., Wadden, K.P., Campbell, K.L. & Boyd, L.A. (2016) High-Intensity Aerobic Exercise Enhances Motor Memory Retrieval. *Med Sci Sports Exerc*, **48**, 2477-2486.

Marin Bosch, B., Bringard, A., Ferretti, G., Schwartz, S. & Igloi, K. (2017) Effect of cerebral vasomotion during physical exercise on associative memory, a near-infrared spectroscopy study. *Neurophotonics*, **4**, 041404.

Marinelli, L., Quartarone, A., Hallett, M., Frazzitta, G. & Ghilardi, M.F. (2017) The many facets of motor learning and their relevance for Parkinson's disease. *Clin Neurophysiol*, **128**, 1127-1141.

Murillo-Rodriguez, E., Desarnaud, F. & Prospero-Garcia, O. (2006) Diurnal variation of arachidonoylethanolamine, palmitoylethanolamide and oleoylethanolamide in the brain of the rat. *Life Sci*, **79**, 30-37.

Pereira, A.C., Huddleston, D.E., Brickman, A.M., Sosunov, A.A., Hen, R., McKhann, G.M., Sloan, R., Gage, F.H., Brown, T.R. & Small, S.A. (2007) An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 5638-5643.

Poldrack, R.A., Sabb, F.W., Foerde, K., Tom, S.M., Asarnow, R.F., Bookheimer, S.Y. & Knowlton, B.J. (2005) The neural correlates of motor skill automaticity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **25**, 5356-5364.

Pontifex, M.B., Parks, A.C., O'Neil, P.C., Egner, A.R., Warning, J.T., Pfeiffer, K.A. & Fenn, K.M. (2014) Poorer aerobic fitness relates to reduced integrity of multiple memory systems. *Cogn Affect Behav Neurosci*, **14**, 1132-1141.

Quercioli, A., Montecucco, F., Pataky, Z., Thomas, A., Ambrosio, G., Staub, C., Di Marzo, V., Ratib, O., Mach, F., Golay, A. & Schindler, T.H. (2013) Improvement in coronary circulatory function in morbidly obese individuals after gastric bypass-induced weight loss: relation to alterations in endocannabinoids and adipocytokines. *Eur Heart J*, **34**, 2063-2073.

Rodriguez-Cueto, C., Hernandez-Galvez, M., Hillard, C.J., Maciel, P., Valdeolivas, S., Ramos, J.A., Gomez-Ruiz, M. & Fernandez-Ruiz, J. (2017) Altered striatal endocannabinoid signaling in a transgenic mouse model of spinocerebellar ataxia type-3. *PloS one*, **12**, e0176521.

Roig, M., Nordbrandt, S., Geertsen, S.S. & Nielsen, J.B. (2013) The effects of cardiovascular exercise on human memory: a review with meta-analysis. *Neuroscience and biobehavioral reviews*, **37**, 1645-1666.

Ros, T., Munneke, M.A., Parkinson, L.A. & Gruzelier, J.H. (2014) Neurofeedback facilitation of implicit motor learning. *Biol Psychol*, **95**, 54-58.

Schapiro, A.C., Reid, A.G., Morgan, A., Manoach, D.S., Verfaellie, M. & Stickgold, R. (2019) The hippocampus is necessary for the consolidation of a task that does not require the hippocampus for initial learning. *Hippocampus*, **29**, 1091-1100.

Schendan, H.E., Searl, M.M., Melrose, R.J. & Stern, C.E. (2003) An FMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. *Neuron*, **37**, 1013-1025.

Scherma, M., Masia, P., Satta, V., Fratta, W., Fadda, P. & Tanda, G. (2019) Brain activity of anandamide: a rewarding bliss? *Acta Pharmacologica Sinica*, **40**, 309-323.

Singh, A.M., Neva, J.L. & Staines, W.R. (2016) Aerobic exercise enhances neural correlates of motor skill learning. *Behavioural brain research*, **301**, 19-26.

Sparling, P.B., Giuffrida, A., Piomelli, D., Rosskopf, L. & Dietrich, A. (2003) Exercise activates the endocannabinoid system. *Neuroreport*, **14**, 2209-2211.

Spielberger CD, G.R., Lushene R, Vagg PR, Jacobs GA (1983) *Manual for the State-Trait Anxiety Inventory (Form Y).* Palo Alto, CA.

Steer, R.A., Ball, R., Ranieri, W.F. & Beck, A.T. (1997) Further evidence for the construct validity of the Beck Depression Inventory-II with psychiatric outpatients. *Psychological Reports*, **80**, 443-446.

Tantimonaco, M., Ceci, R., Sabatini, S., Catani, M.V., Rossi, A., Gasperi, V. & Maccarrone, M. (2014) Physical activity and the endocannabinoid system: an overview. *Cell Mol Life Sci*, **71**, 2681-2698.

Thomas, A., Hopfgartner, G., Giroud, C. & Staub, C. (2009) Quantitative and qualitative profiling of endocannabinoids in human plasma using a triple quadrupole linear ion trap mass spectrometer with liquid chromatography. *Rapid Commun Mass Spectrom*, **23**, 629-638.

Thomas, R., Johnsen, L.K., Geertsen, S.S., Christiansen, L., Ritz, C., Roig, M. & Lundbye-Jensen, J. (2016) Acute Exercise and Motor Memory Consolidation: The Role of Exercise Intensity. *PloS one*, **11**, e0159589.

Thompson, J.A. & Perkel, D.J. (2011) Endocannabinoids mediate synaptic plasticity at glutamatergic synapses on spiny neurons within a basal ganglia nucleus necessary for song learning. *J Neurophysiol*, **105**, 1159-1169.

Thompson, V.A. & Campbell, J.I.D. (2004) A power struggle: Between- vs. within-subjects designs in deductive reasoning research. *Psychologia*, **47**, 277-296.

Uysal, N., Tugyan, K., Kayatekin, B.M., Acikgoz, O., Bagriyanik, H.A., Gonenc, S., Ozdemir, D., Aksu, I., Topcu, A. & Semin, I. (2005) The effects of regular aerobic exercise in adolescent period on hippocampal neuron density, apoptosis and spatial memory. *Neurosci Lett*, **383**, 241-245.

Valenti, M., Vigano, D., Casico, M.G., Rubino, T., Steardo, L., Parolaro, D. & Di Marzo, V. (2004) Differential diurnal variations of anandamide and 2-arachidonoyl-glycerol levels in rat brain. *Cell Mol Life Sci*, **61**, 945-950.

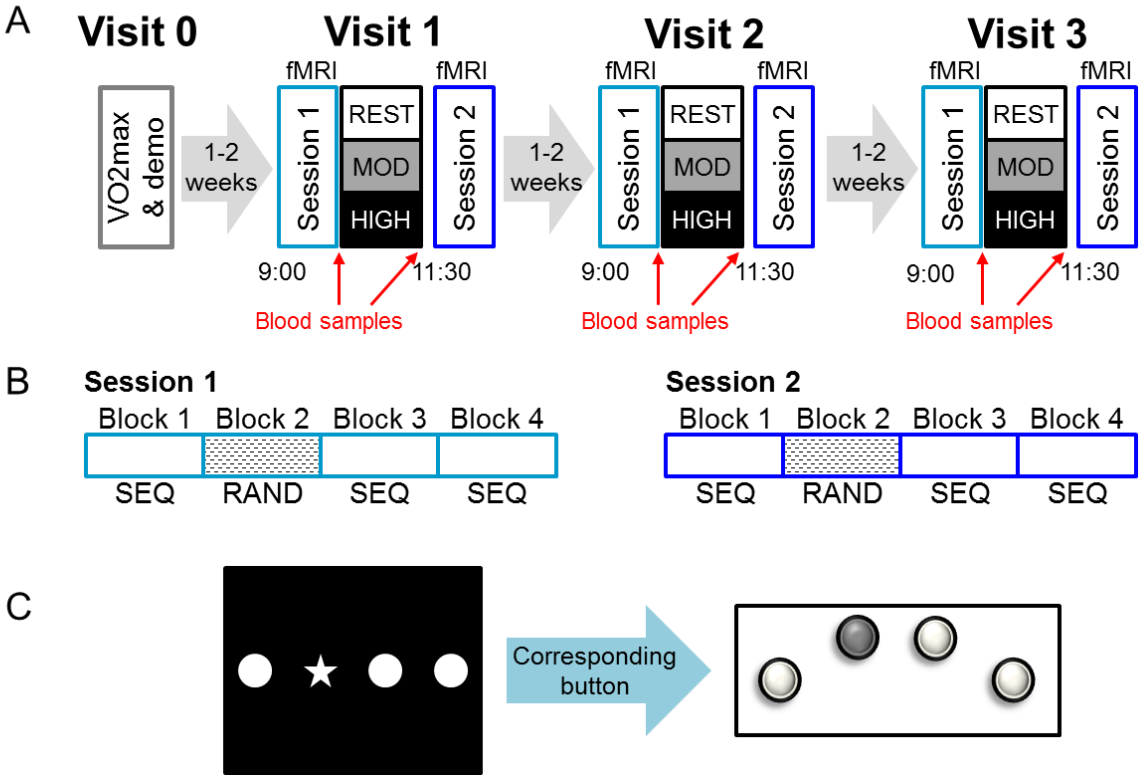
van Dongen, E.V., Kersten, I.H., Wagner, I.C., Morris, R.G. & Fernandez, G. (2016) Physical Exercise Performed Four Hours after Learning Improves Memory Retention and Increases Hippocampal Pattern Similarity during Retrieval. *Curr Biol*, **26**, 1722-1727.

Wenderoth, N., Debaere, F., Sunaert, S. & Swinnen, S.P. (2005) The role of anterior cingulate cortex and precuneus in the coordination of motor behaviour. *Eur J Neurosci*, **22**, 235-246.

Wu, C.W., Chen, Y.C., Yu, L., Chen, H.I., Jen, C.J., Huang, A.M., Tsai, H.J., Chang, Y.T. & Kuo, Y.M. (2007) Treadmill exercise counteracts the suppressive effects of peripheral lipopolysaccharide on hippocampal neurogenesis and learning and memory. *Journal of neurochemistry*, **103**, 2471-2481.

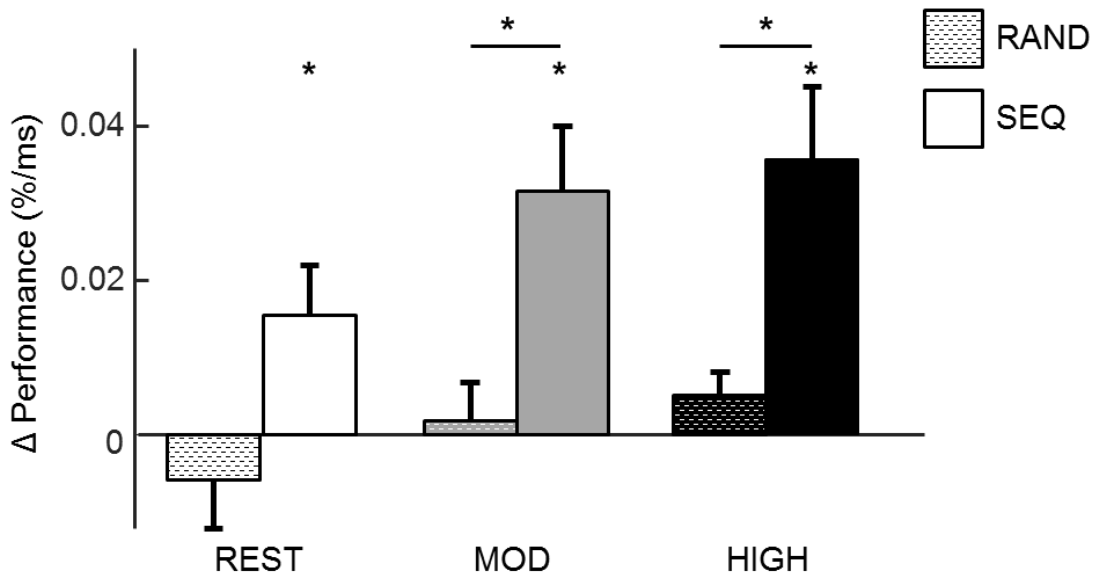
Yanagisawa, H., Dan, I., Tsuzuki, D., Kato, M., Okamoto, M., Kyutoku, Y. & Soya, H. (2010) Acute moderate exercise elicits increased dorsolateral prefrontal activation and improves cognitive performance with Stroop test. *NeuroImage*, **50**, 1702-1710.

Fig 1



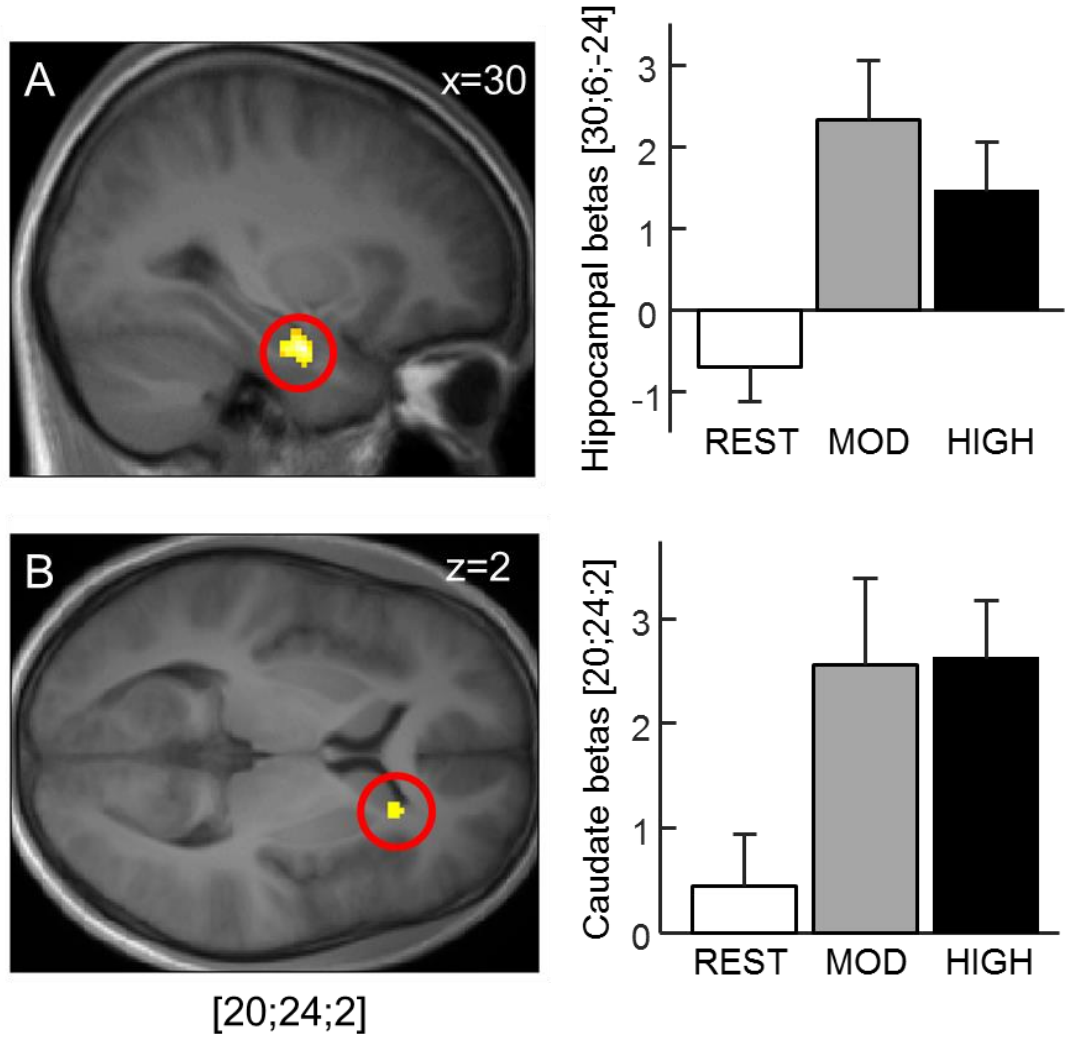
A) Experimental protocol. Participants came to the lab four times, first to do a VO2max session and a demo of the SRTT task, then for 3 experimental visits. Each experimental visit consisted of two fMRI sessions (Session1 and Session 2) separated by a Rest, a Moderate exercise or a High exercise session. Right before and right after the Exercise or Rest session blood samples are taken. Experimental visits took part in the morning between 9AM and 12:30PM. B) Breakdown of the fMRI sessions, both Session 1 and Session 2 are composed of 4 blocks, i.e. 3 sequence blocks and 1 random block (in position 2) showing 4 blocks for each session. A Block is composed of 120 trials for which participants have to press the button corresponding to the stimuli on the screen. Sequence blocks are composed of 10 repetitions of a 12 element-long sequence of key presses whereas random blocks do not contain any repeated sequence but are matched in keypress frequency and inter-trial finger distance to sequence blocks. C) Visual stimulus with corresponding action by participants using the left hand.

Fig 2



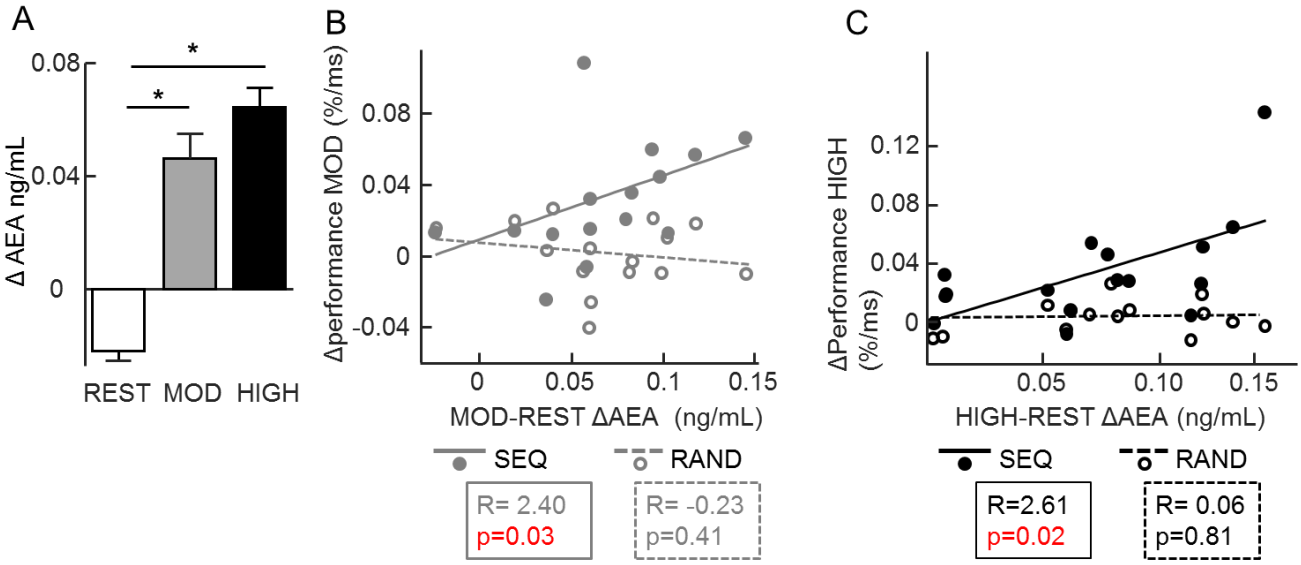
Behavioral results. Performance was defined as mean accuracy (in %) over reaction time (in ms) for both Session 1 and Session 2 of each Exercising condition. Δefficiency is the difference in performance (Δ) from session 1 to session 2. There is a main effect of Exercising condition and of Block type. Post-hoc analyses reveal differences between random and sequence blocks after both moderate and high intensity exercise conditions but not after rest condition.

Fig 3



left: fMRI activations in A) right hippocampus (peak at [30; -6; -24]) and B) right caudate nucleus (peak at [20; 24; 2]) during Session 2 of the SRTT task after moderate and high intensity Exercising Conditions versus the rest Exercising Condition, with reaction times as trialwise parametric modulator. Right: betas for the fMRI activations. All reported activations survive SVC correction for a small volume defined from the AAL atlas in the WFU PickAtlas toolbox (Wake Forest University School of Medicine) fpr SPM12. For display purposes, activations are thresholded at $p<0.005$.

Fig 4



A) Increased Anandamide level (AEA) after moderate and high physical exercise compared to after rest. For all Exercising Conditions Δ AEA corresponds to the difference in AEA between the second blood sample taken after exercise or rest and the first blood sample taken before exercise or rest.

B) Correlation between the increase in performance from Session 1 to Session 2 (Δ performance) for the moderate intensity exercising condition and the increase in Anandamide levels from rest to moderate Exercising conditions (MOD-REST ΔAEA) for the sequence and the random Blocks. Correlation is significant in sequence Blocks but not in the random Blocks.

C) Correlation between the increase in performance from Session 1 to Session 2 (Δ performance) for the high intensity exercising condition and the increase in Anandamide levels from rest to high Exercising conditions (HIGH-REST ΔAEA) for the sequence and the random Blocks. Correlation is significant in the sequence Blocks but not in the random Blocks.

Main effect during sequence blocks								
Cluster size	**Cluster p (unc)**	**Peak T**	**Peak Z**	**Peak p (unc)**	**x**	**y**	**z**	**Brain Region**
465	2.43E-05	9.087	5.122	1.51E-07	-26	-54	-22	Left cerebellum exterior
507	1.29E-05	8.851	5.062	2.07E-07	-22	-56	-50	Left cerebellum exterior
1805	4E-12	8.122	4.864	5.74E-07	36	-26	46	Right precentral gyrus
422	4.72E-05	6.402	4.308	8.25E-06	-10	0	54	Left precentral gyrus
182	0.003	5.426	3.918	4.47E-05	38	-64	-4	Right inferior occipital gyrus
70	0.049	5.080	3.763	8.4E-05	16	2	50	Right supplementary motor cortex
83	0.034	5.033	3.741	9.15E-05	-34	16	12	Left frontal operculum
Main effect during random blocks								
1058	6.34E-10	8.297	4.914	4.47E-07	42	-10	64	Right precentral gyrus
129	0.005	7.526	4.687	1.38E-06	-32	22	6	Left anterior insula
356	3.55E-05	6.754	4.434	4.63E-06	-20	-54	-46	Left cerebellum exterior
33	0.123	6.321	4.278	9.44E-06	34	-42	-48	Right cerebellum exterior
102	0.012	6.135	4.207	1.29E-05	34	22	8	Right frontal operculum
14	0.306	5.295	3.860	5.66E-05	22	-64	60	Right superior parietal lobule
11	0.365	4.875	3.667	0.000123	-54	-20	30	Left postcentral gyrus
35	0.113	4.721	3.592	0.000164	50	6	26	Right precentral gyrus
31	0.133	4.708	3.586	0.000168	-20	-8	52	Left precentral gyrus
84	0.020	4.559	3.512	0.000223	30	-40	42	Right superior parietal lobule
Conjunction analysis								
354	0.0004	6.540	5.053	2.17E-07	-20	-54	-50	left cerebellum
1065	6.84E-08	6.077	4.813	7.45E-07	38	-14	64	right precentral gyrus
97	0.035	5.304	4.376	6.05E-06	-32	18	10	bilateral frontal operculum
90	0.042	5.303	4.375	6.06E-06	34	20	10	
24	0.268	4.454	3.840	6.16E-05	32	-42	-50	Bilateral cerebellum exterior
118	0.022	4.439	3.830	6.41E-05	-24	-52	-22	
34	0.191	4.012	3.536	0.0002	-8	2	52	left supplementary motor
Only sequence blocks: Exercise>rest								
1263	1.53E-12	8.628765	5.003996	2.81E-07	-18	-68	24	left precuneus
159	0.001078	5.228469	3.830734	6.39E-05	-10	-80	10	left calcarine cortex
7	0.426586	5.077322	3.761958	8.43E-05	22	-46	-52	right cerebellum exterior
90	0.00932	5.003377	3.727626	9.66E-05	20	-60	22	right precuneus
Only random blocks: Exercise>rest parametric modulator : inverse reaction time (-RT)								
10	0.280	5.39	3.90	4.74E-05	-42	-86	-4	Left inferior occipital gyrus

Only sequence blocks: Exercise>rest parametric modulator : inverse reaction time (-RT)									
Cluster size	**Cluster p(unc)**	**Peak T**	**Peak Z**	**Peak p (unc)**	**SVC p-value**	**x**	**y**	**z**	**Brain Region**
88	0.006	6.822	4.457	4.15E-06	0.002	30	-6	-24	**Right hippocampus**
27	0.099	5.219	3.826	6.5E-05	0.02	22	24	6	**Right caudate nucleus**